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INTRODUCTION: Mucins form a large family of high-molecular weight glycoproteins that protect and hydrate the interface between tissues and the exterior environment [1]. The general mucin structure of non-glycosylated C- and N-termini and a highly glycosylated mid-part has inspired mucins to be paralleled with co-block polymers and this has led to a somewhat simplistic interpretation of mucin interactions with surfaces [2,3]. Biologically, however, mucins are not simple amphiphilic copolymers. Their conformation at the interface of water/hydrophobic surface is likely to be much more complex and heterogeneous than pictured in this simplistic view. There is therefore need for more refined understanding on the conformation of mucins on surfaces. We have in this study used ligand-specific biomolecular probes to study the surface interactions of bovine submaxillary mucin (BSM).

METHODS: Plate assays were performed on 96-well plates with hydrophobic surface (Brand, Wertheim, Germany). BSM (Sigma Aldrich, St. Louis, MO; purified as described in [4]) was let to spontaneously adsorb to the well surfaces. BSM conformation on the surface was analyzed with enzyme-linked immuno and lectin assays. A C-terminus specific Anti-MUC19 (Abcam, Cambridge, UK) was used in the immuno assay and wheat germ agglutinin (WGA; Sigma Aldrich) and peanut agglutinin (PNA; Sigma Aldrich) in the lectin assays. Protein concentration on the surface was tested with a BCA assay (Sigma Aldrich). Bulk conformation of BSM was studied using circular dichroism spectroscopy (Chirascan, Applied Photophysics Ltd, Surrey, UK) and size distribution using dynamic light scattering (Malvern Instruments Ltd, Worcestershire, UK).

RESULTS: While the amount of BSM on the well surfaces increased proportionally to the amount of BSM in the bulk solution, the anti-BSM (MUC19) showed a drastic variation in binding to BSM as a function of BSM concentration on the surface. Similar non-linear response to BSM concentration was also seen with WGA lectin assay but not with PNA lectin assay. Sodium chloride was found to influence the accessibility of WGA binding sites in BSM, but not PNA binding sites. Bulk BSM concentration had also an influence on BSM conformation; with an increasing concentration, a spectral shift towards more random conformation was observed.

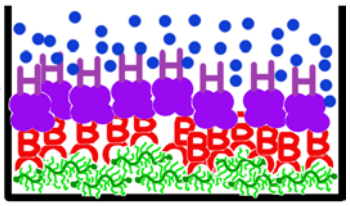

Fig. 1: Schematic presentation of the plate assays used for studying BSM on the microtiter well surface. Green: BSM, red: biotinylated lectin or antibody, streptavidin-conjugated HRP, blue: substrate for HRP.

DISCUSSION & CONCLUSIONS: The non-linear response of the antibody-based immuno assay suggests the C-termini of BSM to become less accessible for binding as the surface concentration of BSM is increased. Similarly, availability of WGA binding sites is decreasing by increasing BSM surface concentration, indicating concentration-dependent changes in BSM conformation on the surface. Effect of sodium chloride on availability of WGA but not PNA binding sites on surface-bound BSM indicates that electrostatic forces have an effect on concentration-conformation relationship of BSM. We suggest a model for concentration-dependent conformational changes of BSM both in bulk solution on a hydrophobic surface; in bulk, coiling of BSM molecules tightens as the concentration of BSM is increased. Similarly, at low surface concentrations BSM molecules are free to lie on the surface but at higher surface concentrations they are forced, due to steric and electrostatic repulsion, to curl up in loop-like conformations to facilitate adsorption of more BSM molecules.

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